

## Influence of Periodizing Dietary Carbohydrate on Iron Regulation and Immune Function in Elite Triathletes

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## 1 Abstract

2 Sleeping with low carbohydrate (CHO) availability is a dietary strategy that may enhance training  
3 adaptation. However, the impact on an athlete's health is unclear. This study quantified the effect of  
4 a short-term "sleep-low" dietary intervention on markers of iron regulation and immune function in  
5 athletes. In a randomized, repeated measures design, 11 elite triathletes completed two four-day  
6 mixed cycle-run training blocks. Key training sessions were structured such that a high-intensity  
7 training (HIT) session was performed in the field on the afternoon of days 1 and 3, and a low-  
8 intensity training (LIT) session on the following morning in the laboratory (days 2 and 4). The  
9 ingestion of CHO was either divided evenly across the day (HIGH), or restricted between the HIT  
10 and LIT sessions, so that the LIT session was performed with low CHO availability (LOW). Venous  
11 blood and saliva samples were collected prior to and following each LIT session and analyzed for  
12 interleukin-6 (IL-6), hepcidin-25 and salivary immunoglobulin-A (s-IgA). Concentrations of IL-6  
13 increased acutely after exercise ( $p<0.001$ ), but did not differ between dietary conditions or days.  
14 Hepcidin-25 increased 3 h post-exercise ( $p<0.001$ ), with the greatest increase evident after the LOW  
15 trial on day 2 ( $2.5\pm0.9$  fold increase $\pm 90\%$  CL). The s-IgA secretion rate did not change in response  
16 to exercise, however, was highest during the LOW condition on day 4 ( $p=0.046$ ). There appears to  
17 be minimal impact on markers of immune function and iron regulation when acute exposure to low  
18 CHO availability is undertaken with expert nutrition and coaching input.

19

20 **Key words:** sleep low; train low; hepcidin.

## 21 **Introduction**

22 Acute carbohydrate (CHO) restriction around exercise can enhance molecular adaptations to training  
23 by augmenting cell signaling, gene expression, enzyme activity and lipid oxidation (Impey et al.,  
24 2018). These outcomes inform nutritional guidelines, which advise athletes to strategically approach  
25 CHO periodization (Jeukendrup, 2017). One strategy is the “sleep low” sequence (Burke et al.,  
26 2018), which involves commencing a high-intensity training session in the evening with high CHO  
27 availability, before restricting CHO intake at the subsequent meal and overnight. This protocol  
28 supports high quality training for the first session, augmented by an enhanced period of cellular  
29 signaling related to delayed restoration of muscle glycogen. The following morning’s submaximal  
30 exercise session is performed under conditions of low muscle and liver glycogen, promoting higher  
31 reliance on fat oxidation, greater metabolic stress and cellular adaptation (Bartlett et al., 2015).  
32 While careful integration of such strategies can enhance adaptation and performance (Marquet et al.,  
33 2016), the potential implications on athlete health are relatively unknown. Accordingly, concern  
34 centers on transient immunodepression associated with endurance exercise, which might otherwise  
35 be attenuated by CHO ingestion. Current research shows that low or declining levels of salivary  
36 immunoglobulin-A (s-IgA) are associated with increased risk of upper respiratory illness (URI)  
37 (Bishop & Gleeson, 2009); however, both increases (McKay et al., 2018) and decreases (Louis et al.,  
38 2016) in s-IgA levels are reported in response to a 3-week periodized CHO intervention; thus, the  
39 acute impact of this strategy is unclear.

40

41 Training with low CHO availability can also increase post-exercise concentrations of the  
42 inflammatory cytokine interleukin-6 (IL-6) (Hennigar et al., 2017), which may have downstream  
43 implications for the iron-regulatory hormone hepcidin (Badenhorst et al., 2015). Adherence to a low  
44 CHO diet (3 g/kg) for 24 h can amplify the immediate post-exercise IL-6, and 3 h post-exercise  
45 hepcidin response as compared to high (10 g/kg) CHO availability (Badenhorst et al., 2015).

Increases in hepcidin levels occur ~3-6 h post-exercise (Peeling et al., 2009), potentially reducing dietary iron absorption and macrophage iron recycling (Nemeth et al., 2004). Despite the potential long-term implications to athlete health and performance, studies exploring these effects in elite athletes are lacking. Therefore, we quantified the effect of a sleep-low protocol in the daily training environment on markers of inflammation, iron regulation and immune function in elite triathletes. Given that exercise modality can also influence the post-exercise inflammatory response (Nieman et al., 1998), we compared responses between exercise modalities.

53

## 54 **Methods**

### 55 **Participants**

Four male ( $22.5 \pm 3.0$  y,  $64.3 \pm 4.1$  kg,  $39 \pm 9$  mm for sum of 7 skinfolds,  $74.8 \pm 6.2$  and  $76.6 \pm 1.6$  ml·kg<sup>-1</sup>·min<sup>-1</sup> for cycle and treadmill VO<sub>2peak</sub> respectively; mean±SD) and seven female ( $26.4 \pm 2.0$  y,  $56.4 \pm 5.4$  kg,  $62 \pm 20$  mm for sum of 7 skinfolds,  $66.2 \pm 5.1$  and  $65.3 \pm 4.4$  ml·kg<sup>-1</sup>·min<sup>-1</sup> for cycle and treadmill VO<sub>2peak</sub>, respectively) triathletes with >18 months of elite training history were recruited. Skinfold measurements were undertaken by an accredited anthropometrist according to International Society for the Advancement of Kinanthropometry standards. Athletes were free from injury, illness, or iron deficiency (serum ferritin >30 µg·L<sup>-1</sup>); none were taking iron supplements during the testing period. Written informed consent was obtained prior to participation. Approval was granted by the Australian Institute of Sport (AIS) Ethics Committee.

65

This study was conducted during an AIS training camp over a 4-week period between two World Triathlon Series events. In a randomized, crossover design athletes completed two 4-day experimental trials with high CHO availability (HIGH) or adopting an alternate day sleep-low protocol (LOW) (see Figure 1). Athletes completed familiarization tests prior to intervention commencement, from which, workloads for subsequent testing sessions were derived. Tests included a 4-min maximal cycling test to determine cycling VO<sub>2peak</sub> (Gore et al., 1998) and mean maximal

power (MMP), and a graded treadmill test to determine  $\text{VO}_{2\text{peak}}$  and running velocity at  $\text{VO}_{2\text{peak}}$  ( $v\text{VO}_2$ ), as described elsewhere (Tanner & Gore, 2013). Additionally, a venous blood sample was collected from female athletes to measure sex hormone concentrations and determine menstrual phase. Athletes were subsequently pair-matched based on performance measures and menstrual phase, before being randomized into their first dietary condition.

## **Nutrition Interventions**

During experimental trials, a standardized daily CHO intake of 6 (females) or 8  $\text{g}\cdot\text{kg}^{-1}$  body mass (males) was consumed, with differences attributed to disparities in lean body mass and habitual CHO intake between sexes. Athletes completed a directed daily dietary record and ate in a dining hall setting where all meal options were prepared from standardized recipes, allowing free-living with a high degree of dietary control. Athletes were provided with individualized CHO targets for each meal, and were guided to self-select food and fluid to accommodate dietary preferences while meeting dietary targets. A research team member attended all meals to ensure compliance, before undertaking subsequent analysis (Food Works 8 Professional program; Xyris Software Australia Pty Ltd, Australia). Although each athlete's individual CHO intake was matched across experimental trials, CHO intake was periodized differently across days according to trial allocation. During LOW, a high intensity training (HIT) session was performed in the afternoon to deplete muscle glycogen stores on days 1 and 3 (Table 1). CHO intake was scheduled predominantly pre HIT with post-session consumption restricted ( $<0.5 \text{ g}\cdot\text{kg}^{-1}$ ) ahead of a low intensity aerobic session (LIT) the following morning. During HIGH, the CHO intake was scheduled evenly throughout the day and following the HIT session to facilitate overnight recovery of muscle glycogen stores.

## **Training**

Athletes completed an individualized training program, involving 3-4 daily sessions of running, swimming, cycling and resistance training (Table 1). Training was structured to include a field-based

HIT session on the afternoons of day 1 and 3, and a laboratory-based LIT session the following morning (day 2 and 4). This design achieved 2 repetitions of the sleep-low protocol within each 4-day period. This protocol incorporated mixed-mode training practices for elite triathletes, with cycling sessions performed on days 1 and 2 and running sessions on days 3 and 4.

### *High Intensity Training Sessions*

To ensure high external validity and account for the proximity of the upcoming international event, all HIT sessions were individually prescribed and led by an international-level coach who understood the study goals. During the HIT cycling session (day 1), athletes completed an interval-based session (~45 min) at an outdoor velodrome on a fixed-gear bike, in addition to cycling to and from the training venue (LOW: 31.2±5.1 km, 76.1±9.0 min return; HIGH 28.8±0.9 km, 70.5±1.9 min return). The HIT running session (day 3) was interval-based, prescribed from time and/or distance to equal ~10 km (LOW: 53.2±5.5 min, mean heart rate (HR) 166±6 bpm; HIGH: 53.2±9.5 min, mean HR 165±11 bpm).

### *Low Intensity Training Sessions*

Athletes reported to the laboratory for LIT sessions at a standardized time in an overnight fasted and rested state. A 4 mL baseline venous blood sample was collected from an antecubital vein before athletes consumed a snack in accordance with their dietary allocation (1.5 v 0.0 g·kg<sup>-1</sup> CHO for HIGH and LOW, respectively); 25 min later, they provided a saliva sample. Thirty min post-snack, athletes commenced their planned LIT task. The 60 min cycle trial (day 2) was performed in the laboratory on the athlete's own bicycle mounted to a stationary cycle ergometer. The 45 min running trial (day 4) was conducted as a hybrid laboratory-field test, with the first and final 5 min completed on a motorized treadmill, and the remainder completed on a flat, outdoor course. The first and final 5 min of each trial were completed at 65% MMP (cycle) or  $\dot{V}O_2$  (run) with the remaining distance

completed at ~55% MMP or  $\dot{V}O_2$ . During HIGH, athletes consumed 0.5 g·kg<sup>-1</sup> CHO during exercise, while water was consumed during LOW. Mean HR and respiratory exchange ratio were determined for the final 5 min of each session, while venous blood and saliva samples were collected immediately and 15 min post-exercise, respectively. Athletes received a whey protein shake immediately following saliva collection (0.3 g·kg<sup>-1</sup> whey protein) and a CHO-rich breakfast 1 h post-exercise (2 g·kg<sup>-1</sup> CHO) to ensure recovery and avoid hunger. Venous blood samples were collected 1 and 3 h post-exercise.

## Experimental Procedures

### *Blood Sampling*

Venous blood samples were collected into 4 mL SST gel separator tubes and left to clot for 30 min before centrifugation (2200 G for 10 min). Serum was divided into 1 mL cryotubes and frozen at -80°C until batch analysis of iron, serum ferritin, IL-6 and hepcidin-25 concentrations. Pre-exercise serum iron and ferritin concentrations were quantified via a COBAS Integra 400 automated biochemistry analyzer (Roche Diagnostics, Switzerland). IL-6 concentrations were analyzed in duplicate pre-, post-, 1 h and 3 h post-exercise using an ELISA (Quantikine HS, R&D Systems, Minneapolis, USA; CV=5.7%). Hepcidin-25 measurements were performed on serum obtained pre- and 3 h post-exercise ([www.hepcidinanalysis.com](http://www.hepcidinanalysis.com), Nijmegen, The Netherlands) using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (Kroot et al., 2010; Swinkels et al., 2008). When values were below the lower limit of detection of 0.5 nM, this value divided by the square root of 2 was used (Croghan & Egeghy, 2003).

### *Saliva Sampling*

Prior to, and following each LIT session, an unstimulated saliva sample was obtained 10 min after a 40 ml water mouth rinse. Prior to sample collection, a >20 min window where no CHO was ingested

was employed to minimize potential sample contamination. Samples were collected using a saliva collection aid (Salimetrics, State College, PA) over a timed 2 min period into 2 ml cryotubes and subsequently weighed. Assuming a saliva density of  $1.00 \text{ g}\cdot\text{mL}^{-1}$  (Cole & Eastoe, 1988), flow rate ( $\text{mL}\cdot\text{min}^{-1}$ ) was calculated by multiplying saliva volume by collection time. Samples were centrifuged at 1500 G for 15 min, before s-IgA concentrations were analyzed in duplicate (EIA kits, Salimetrics, State College, PA; CV=5.8%). Secretion rates ( $\mu\text{g}\cdot\text{min}^{-1}$ ) were calculated by multiplying the salivary flow rate by s-IgA concentration.

#### *Statistical Analysis*

Data were analyzed in SPSS (version 25.0, IBM, USA) using a linear mixed model and presented as mean $\pm$ standard deviation. A random intercept for subjects was included to adjust for baseline levels and inter-individual homogeneity. Initial models included all possible interactions, but non-significant interaction terms were dropped from the models for ease of interpretation.

#### **Results**

Self-recorded dietary intakes (Table 2) did not differ for energy, CHO, protein, fat and iron between treatments, however timing of CHO intake across the day was different. As intended, CHO intake before the HIT session on days 1 and 3 was markedly lower in HIGH ( $4.6\pm0.6 \text{ g}\cdot\text{kg}^{-1}$ ) than LOW ( $6.7\pm1.1 \text{ g}\cdot\text{kg}^{-1}$ ;  $p<0.001$ ), while intakes after HIT were greater in HIGH ( $2.6\pm0.7 \text{ g}\cdot\text{kg}^{-1}$ ) than LOW ( $0.3\pm0.1 \text{ g}\cdot\text{kg}^{-1}$ ;  $p<0.001$ ).

Measures of training performance from the morning LIT sessions showed no differences in power output ( $F(1,19)=0.11$ ,  $p=0.741$ ) or running speed ( $F(1,18)=0.48$ ,  $p=0.497$ ) between trials (Table 3). Moderately lower RER ( $-4.2\%$ ;  $p<0.001$ ) and higher HR ( $3.8\%$ ;  $p=0.053$ ) occurred during LOW, compared to HIGH. Moderate increases in HR ( $4.7\%$ ;  $p=0.019$ ) and large increases in RER ( $8.4\%$ ;  $p<0.001$ ) were evident during cycling compared to running.



Resting serum ferritin levels were  $56 \pm 21$  and  $52 \pm 16$   $\mu\text{g}\cdot\text{L}^{-1}$  during LOW, and  $50 \pm 17$  and  $50 \pm 15$   $\mu\text{g}\cdot\text{L}^{-1}$  during HIGH (cycle and run, respectively). No differences in serum ferritin were seen between dietary interventions or exercise modalities ( $p > 0.05$ ). Concentrations of IL-6 (Figure 2) gradually increased from pre- to 3 h post-exercise during all trials ( $F(3,161) = 28.5$ ,  $p < 0.001$ ). No clear differences between exercise modalities or dietary interventions were evident ( $p > 0.05$ ). Finally, hepcidin-25 increased substantially from pre- to 3 h post-exercise ( $F(1,78) = 20.2$ ,  $p < 0.001$ ), with a greater increase seen after cycling than running ( $F(1,78) = 4.2$ ,  $p = 0.043$ ). Furthermore, the increase in hepcidin-25 was greatest during cycling when adhering to LOW than HIGH ( $F(1,78) = 5.2$ ,  $p = 0.026$ ).

Markers of mucosal immunity (Table 4) showed that s-IgA concentrations increased post-exercise (17-68%) during all trials ( $F(1,81) = 8.6$ ,  $p = 0.004$ ) but were not different between exercise modalities or dietary interventions ( $p > 0.05$ ). Salivary flow rates decreased consistently (19-39%) post-exercise ( $F(1,81) = 4.3$ ,  $p = 0.042$ ), with trivial differences between exercise modalities ( $F(1,81) = 0.70$ ,  $p = 0.404$ ) and dietary interventions ( $F(1,81) = 0.11$ ,  $p = 0.917$ ). s-IgA secretion rates were greater during the LOW run than all other trials ( $F(1,83) = 4.1$ ,  $p = 0.046$ ). No other clear differences in s-IgA secretion rates were apparent.

185

## 186 Discussion

This study examined the influence of a sequenced periodization of CHO availability (“sleep low”) on markers of inflammation, iron metabolism and immune function in elite athletes. No differences in the IL-6 response to exercise between trials or dietary conditions were evident, however, the 3 h post-exercise hepcidin response was highest during the LOW cycling trial compared to all other trials. Exercise had an acute effect on mucosal immune markers, but differences between trials or dietary conditions were trivial. It appears that acute rescheduling of daily CHO intake to delay glycogen restoration from previous exercise sessions to create low CHO availability for a subsequent

194 session of low intensity exercise causes minimal perturbations to the immune system or iron  
195 regulation in elite triathlon training settings.

196

197 We investigated the interactions between exercise modality and CHO availability, by exposing run  
198 and cycling sessions of equal metabolic stress to two different scenarios of CHO availability.  
199 However, HR and RER measures during LIT sessions were higher during cycling than running,  
200 showing our failure to accurately match the exercise intensity between modalities. We attribute this  
201 shortcoming to differences between a graded exercise test (run) and a 4 min MMP test (cycle) for  
202 prescribing exercise intensity. While both protocols should elicit a similar  $\text{VO}_{2\text{peak}}$  (Gore et al.,  
203 1998), the lack of cumulative fatigue during the shorter protocol (4 min MMP test) likely resulted in  
204 a higher power at  $\text{VO}_{2\text{peak}}$  than during the incremental test. As such, the prescribed cycling power  
205 occurred at a higher intensity than the equivalent target pace for running. Therefore, while direct  
206 comparison of modalities is not entirely appropriate, this study provides insights on the impact of  
207 exercise intensity to iron regulation and the immune system when CHO availability is manipulated.  
208 Importantly, our dietary intervention appears to have been successful, with an increased HR and  
209 decreased RER achieved during LOW compared to HIGH, despite the similarity of external  
210 workloads. Differences can be attributed to increased metabolic stress and fat utilization in tandem  
211 with reductions in endogenous and exogenous CHO availability (Yeo et al., 2008).

212

213 Increases in s-IgA concentration and decreases in salivary flow rate were evident after each LIT  
214 session, which confirms previous findings (Li & Gleeson, 2004; McKay et al., 2018), where  
215 exercise-induced dehydration and activation of the sympathetic nervous system decreased salivary  
216 flow rate to elicit a concentrating effect on s-IgA levels (Bishop et al., 2000). When these factors  
217 were accounted for, there were no differences in secretion rates between all four LIT trials. It is  
218 possible that the low intensity, short duration exercise bout (45-60 min) failed to achieve a

sufficiently large exercise stress to elicit alterations to immune function. An increase in s-IgA secretion rate was evident from day 2 to day 4 in the LOW condition only, suggestive of enhanced mucosal defense in response to low CHO availability. Indeed, we previously reported increases in resting s-IgA secretion rates while athletes completed a 3-week block of training involving periodized CHO availability (McKay et al., 2018). Conversely, 3-weeks of an alternate day “sleep low” intervention yielded a small decrease in resting s-IgA levels in trained triathletes (Louis et al., 2016), however was deemed unlikely to be clinically meaningful and did not impact illness rates. Collectively, it appears that acute ‘sleep-low’ approaches to training are not detrimental to the immune system when the training performed with low CHO availability is of short duration and low intensity.

Despite inducing differences in metabolism and fuel utilization, training with low CHO availability did not increase the inflammatory response to exercise. Although IL-6 levels can be increased after exercise performed with low muscle glycogen stores (Steensberg et al., 2001), our intervention may not have sufficiently depleted muscle glycogen stores to create functional differences between conditions. Specifically, the HIT sessions performed on days 1 and 3 were coach-designed to balance the study goals with up-coming international competition, and were intended to maximize performance rather than deplete glycogen stores. Conversely, it is possible that the high daily training loads of our triathlete population created intrinsic glycogen depletion patterns that overshadowed the differential effects of timing of dietary intake, particularly since total CHO intake was similar. Furthermore, since the magnitude of the IL-6 increase is exercise duration- and intensity-dependent (Fischer, 2006), the small IL-6 response to a short, LIT session reduces the potential for detection of differences.

Despite the lack of differences in IL-6 concentrations between trials, there was a greater increase in post-exercise hepcidin concentrations following the LOW-LIT cycle session. This is an unexpected outcome if an augmented IL-6 response is considered the primary mechanism for post-exercise hepcidin changes. However, we recently reported that although an increase in IL-6 is necessary to invoke this change, its contribution to the magnitude of hepcidin response in exercise settings appears small (Peeling et al., 2017; McKay et al., 2019) and other factors are likely to be involved. Exercise intensity may be a regulating factor since the augmented response was only evident in the cycling session. These outcomes strengthen the evidence for the intentional planning of LIT sessions during periods of low CHO availability, since this approach appears to minimize any potential impact on iron metabolism.

Since maintenance of good health underpins the consistency of training and optimal performance (Raysmith & Drew, 2016), it is important to understand the impact of emerging sports nutrition strategies on outcomes such as inflammation, iron metabolism and the immune system. The highly applied nature of this study created limitations to experimental control in the general prescription of the training program, as well as catering for individual athlete requirements. However, the fusion of laboratory-based testing within the daily training environment gives our results strong ‘real-world’ credibility, and outcomes are highly relevant for elite-level triathletes. While we have examined the acute impact of this dietary strategy, the potential long-term effects of repeated bouts of CHO restriction merit further investigation. Our findings suggest periodizing CHO availability to promote adaptation throughout the training cycle had limited impact on alterations to markers of athlete health, at least when occasional and planned exposure to low CHO availability was undertaken with expert nutrition and coaching input. Accordingly, when sessions performed with low CHO availability are low intensity and short in duration, there is likely only minimal impact on exercise-induced alterations in immune function and iron regulation.

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## 279    **References**

- 280    Badenhorst, C., Dawson, B., Cox, G., Laarakkers, C., Swinkels, D., & Peeling, P. (2015). Acute  
281       dietary carbohydrate manipulation and the subsequent inflammatory and hepcidin responses  
282       to exercise. *European Journal of Applied Physiology*, 115(12), 2521-2530.
- 283    Bartlett, J. D., Hawley, J. A., & Morton, J. P. (2015). Carbohydrate availability and exercise training  
284       adaptation: too much of a good thing? *European Journal of Sport Science*, 15(1), 3-12.
- 285    Bishop, N. C., & Gleeson, M. (2009). Acute and chronic effects of exercise on markers of mucosal  
286       immunity. *Frontiers in Bioscience*, 14, 4444-4456.
- 287    Bishop, N. C., Blannin, A. K., Armstrong, E., Rickman, M., & Gleeson, M. (2000). Carbohydrate  
288       and fluid intake affect the saliva flow rate and IgA response to cycling. *Medicine and Science*  
289       *in Sports and Exercise*, 32(12), 2046-2051.
- 290    Burke, L. M., Hawley, J. A., Jeukendrup, A., Morton, J. P., Stellingwerff, T., & Maughan, R. J.  
291       (2018). Toward a common understanding of diet–exercise strategies to manipulate fuel  
292       availability for training and competition preparation in endurance sport. *International Journal*  
293       *of Sport Nutrition and Exercise Metabolism*, 28(5), 451-463.
- 294    Cole, A. S., & Eastoe, J. E. (1988). *Biochemistry and Oral Biology*. London: Wright.
- 295    Croghan, C., & Egeghy, P. (2003). Methods of dealing with values below the limit of detection using  
296       SAS. *Southern SAS User Group*, 22, 24.
- 297    Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance?  
298       *Exercise Immunology Review*, 12, 6-33.
- 299    Gore, C., Craig, N., Hahn, A., Rice, A., Bourdon, P., Lawrence, S., . . . Parisotto, R. (1998). Altitude  
300       training at 2690m does not increase total haemoglobin mass or sea level VO<sub>2</sub>max in world  
301       champion track cyclists. *Journal of Science and Medicine in Sport*, 1(3), 156-170.
- 302    Hennigar, S. R., McClung, J. P., & Pasiakos, S. M. (2017). Nutritional interventions and the IL-6  
303       response to exercise. *The FASEB Journal*, 31(9), 3719-3728.

- 304 Impey, S. G., Hearn, M. A., Hammond, K. M., Bartlett, J. D., Louis, J., Close, G. L., & Morton, J.  
305 P. (2018). Fuel for the work required: a theoretical framework for carbohydrate periodization  
306 and the glycogen threshold hypothesis. *Sports Medicine*, 48(5), 1031-1048.
- 307 Jeukendrup, A. E. (2017). Periodized nutrition for athletes. *Sports Medicine*, 47(1), 51-63.
- 308 Kroot, J. J., Laarakkers, C. M., Geurts-Moespot, A. J., Grebenchtchikov, N., Pickkers, P., van Ede,  
309 A. E., . . . Sweep, F. C. (2010). Immunochemical and mass-spectrometry-based serum  
310 hepcidin assays for iron metabolism disorders. *Clinical Chemistry*, 56(10), 1570-1579.
- 311 Li, T., & Gleeson, M. (2004). The effect of single and repeated bouts of prolonged cycling and  
312 circadian variation on saliva flow rate, immunoglobulin A and-amylase responses. *Journal of*  
313 *Sports Sciences*, 22(11-12), 1015-1024.
- 314 Louis, J., Marquet, L.-A., Tiollier, E., Bermon, S., Hausswirth, C., & Brisswalter, J. (2016). The  
315 impact of sleeping with reduced glycogen stores on immunity and sleep in triathletes.  
316 *European Journal of Applied Physiology*, 116(10), 1941-1954.
- 317 Marquet, L.-A., Brisswalter, J., Louis, J., Tiollier, E., Burke, L. M., Hawley, J. A., & Hausswirth, C.  
318 (2016). Enhanced endurance performance by periodization of CHO intake: “sleep low”  
319 strategy. *Medicine and Science in Sports and Exercise*, 48(4), 663-672.
- 320 McKay, A. K., Peeling, P., Pyne, D. B., Welvaert, M., Tee, N., Leckey, J. J., . . . Burke, L. M.  
321 (2019). Acute carbohydrate ingestion does not influence the post-exercise iron-regulatory  
322 response in elite keto-adapted race walkers. *Journal of Science and Medicine in Sport*. In  
323 Press. doi: 10.1016/j.jsams.2018.12.015.
- 324 McKay, A. K., Pyne, D. B., Peeling, P., Sharma, A. P., Ross, M. L., & Burke, L. M. (2018). The  
325 impact of chronic carbohydrate manipulation on mucosal immunity in elite endurance  
326 athletes. *Journal of Sports Sciences*, 37(5), 553-559.

- 327 Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., . . . Kaplan, J.  
 328 (2004). Heparin regulates cellular iron efflux by binding to ferroportin and inducing its  
 329 internalization. *Science*, 306(5704), 2090-2093.
- 330 Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D. A., Utter, A., Davis, J. M., . . .  
 331 Butterworth, D. E. (1998). Influence of mode and carbohydrate on the cytokine response to  
 332 heavy exertion. *Medicine and Science in Sports and Exercise*, 30(5), 671-678.
- 333 Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E. T., Swinkels, D. W., & Trinder,  
 334 D. (2009). Effects of exercise on hepcidin response and iron metabolism during recovery.  
 335 *International Journal of Sport Nutrition and Exercise Metabolism*, 19(6), 583-597.
- 336 Peeling, P., McKay, A. K., Pyne, D. B., Guelfi, K. J., McCormick, R. H., Laarakkers, C. M., . . .  
 337 Sharma, A. P. (2017). Factors influencing the post-exercise hepcidin-25 response in elite  
 338 athletes. *European Journal of Applied Physiology*, 117(6), 1233-1239.
- 339 Raysmith, B. P., & Drew, M. K. (2016). Performance success or failure is influenced by weeks lost  
 340 to injury and illness in elite Australian track and field athletes: A 5-year prospective study.  
 341 *Journal of Science and Medicine in Sport*, 19(10), 778-783.
- 342 Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., Hall, G., Saltin, B., & Pedersen, B. K.  
 343 (2001). Interleukin-6 production in contracting human skeletal muscle is influenced by  
 344 pre-exercise muscle glycogen content. *The Journal of Physiology*, 537(2), 633-639.
- 345 Swinkels, D. W., Girelli, D., Laarakkers, C., Kroot, J., Campostrini, N., Kemna, E. H., & Tjalsma,  
 346 H. (2008). Advances in quantitative hepcidin measurements by time-of-flight mass  
 347 spectrometry. *PloS One*, 3(7), e2706.
- 348 Tanner, R., Gore, C. (2013). *Physiological tests for elite athletes 2nd Edition*: Champaign, IL:  
 349 Human Kinetics.



- 350 Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the academy of nutrition and  
351 dietetics, dietitians of Canada, and the American college of sports medicine: Nutrition and  
352 athletic performance. *Journal of the Academy of Nutrition and Dietetics*, 116(3), 501-528.
- 353 Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L., & Hawley, J. A. (2008).  
354 Skeletal muscle adaptation and performance responses to once a day versus twice every  
355 second day endurance training regimens. *Journal of Applied Physiology*, 105(5), 1462-1470.
- 356

**Table 1.** Prescribed training program and carbohydrate (CHO) targets (male athlete; 8.0 g·kg<sup>-1</sup>CHO) for each 4-day experimental trial when adopting a sleep-low (LOW) and sleep high approach (HIGH). HIT: High-intensity training session; LIT: Low-intensity training session; LSD: Long, slow duration session.

	DAY 1		DAY 2		DAY 3		DAY 4	
	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW
Breakfast	2.5 g·kg <sup>-1</sup>	3.5 g·kg <sup>-1</sup>	1.5 g·kg <sup>-1</sup>	0 g·kg <sup>-1</sup>	2.5 g·kg <sup>-1</sup>	3.5 g·kg <sup>-1</sup>	1.5 g·kg <sup>-1</sup>	0 g·kg <sup>-1</sup>
	Run		LIT Cycle		Run		LIT Run	
Session 1	40 min LSD		0.5 g·kg <sup>-1</sup> CHO	0 g·kg <sup>-1</sup> CHO	20 min LSD + 4 x 45 sec builds		0.5 g·kg <sup>-1</sup>	0 g·kg <sup>-1</sup>
Post training Snack	-		2 g·kg <sup>-1</sup> CHO	2 g·kg <sup>-1</sup> CHO	-		2 g·kg <sup>-1</sup>	2 g·kg <sup>-1</sup>
Session 2	Gym 30 mins		-		Gym 30 mins		-	
Session 3	Swim 60 min AT main set		Run 40 min LSD + 6 x 30 sec builds		Swim 60 min AT main set		Cycle 90 min aerobic	
Lunch + Snacks	2.5 g·kg <sup>-1</sup>	4 g·kg <sup>-1</sup>	2 g·kg <sup>-1</sup>	3 g·kg <sup>-1</sup>	2.5 g·kg <sup>-1</sup>	4 g·kg <sup>-1</sup>	2 g·kg <sup>-1</sup>	3 g·kg <sup>-1</sup>
Session 4	HIT Cycle		Swim 60 min Aerobic Pull		HIT Run		Swim 60 min Aerobic Pull	
	0.5 g·kg <sup>-1</sup>	0.5 g·kg <sup>-1</sup>			0.5 g·kg <sup>-1</sup>	0.5 g·kg <sup>-1</sup>		
Dinner	2.5 g·kg <sup>-1</sup>	0 g·kg <sup>-1</sup>	2 g·kg <sup>-1</sup>	3 g·kg <sup>-1</sup>	2.5 g·kg <sup>-1</sup>	0 g·kg <sup>-1</sup>	2 g·kg <sup>-1</sup>	3 g·kg <sup>-1</sup>

**Table 2.** Daily total energy intake, macronutrient concentrations and daily iron content of athletes when adopting a sleep-low (LOW) and sleep high approach (HIGH); Mean ( $\pm$ SD).

		Day 1		Day 2		Day 3		Day 4	
		LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
<b>Energy</b> (kJ·kg <sup>-1</sup> )	Mean	277	279	300	299	267	261	293	287
	SD	38	40	43	37	30	36	39	49
<b>Carbohydrate</b> (g·kg <sup>-1</sup> )	Mean	7.5	7.7	7.7	8.2	6.9	7.1	7.7	8.0
	SD	1.3	1.2	1.4	1.6	1.1	1.1	1.3	1.5
<b>Fat</b> (g·kg <sup>-1</sup> )	Mean	2.3	2.5	2.9	2.7	2.4	2.3	2.8	2.4
	SD	0.5	0.5	0.5	0.4	0.5	0.5	0.6	0.5
<b>Protein</b> (g·kg <sup>-1</sup> )	Mean	3.4	3.1	3.2	3.3	3.1	3.0	3.4	3.4
	SD	0.8	0.7	0.8	0.6	0.7	0.7	1.0	0.6
<b>Iron</b> (mg)	Mean	23.24	24.9	26.2	25.0	23.9	22.4	20.2	21.9
	SD	5.8	7.6	4.0	5.5	5.5	8.4	3.6	5.7

**Table 3.** Internal and external load variables for low intensity sessions when adopting a sleep low (LOW) and sleep high approach (HIGH). Data are presented as mean ( $\pm$ SD). \*indicates values are significantly greater than day 4. #indicates values are significantly lower than HIGH trials.

	Trial	Day 2 - Cycle	Day 4 - Run
<b>55% Intensity</b>	LOW	164 $\pm$ 23	5:15 $\pm$ 0:19
<i>(W OR min/km)</i>	HIGH	164 $\pm$ 22	5:09 $\pm$ 0:12
<b>65% Intensity</b>	LOW	212 $\pm$ 27	4:45 $\pm$ 0:26
<i>(W OR min/km)</i>	HIGH	216 $\pm$ 33	4:45 $\pm$ 0:26
<b>Mean Heart Rate</b>	LOW	158 $\pm$ 9*	151 $\pm$ 13
<i>(bpm)</i>	HIGH	152 $\pm$ 9*	144 $\pm$ 9
<b>RER</b>	LOW	0.84 $\pm$ 0.04 <sup>#*</sup>	0.76 $\pm$ 0.02 <sup>#</sup>
<i>(Respiratory exchange ratio)</i>	HIGH	0.87 $\pm$ 0.03*	0.80 $\pm$ 0.03

**Table 4.** s-IgA concentration, flow rate and secretion rate pre- and post-exercise in athletes adopting a sleep low (LOW) or sleep high (HIGH) dietary approach. Data are presented as Mean ( $\pm$ SD). \* Indicates a significant increase from pre-exercise. # Indicates a significant decrease from pre-exercise. ^ Significantly greater than all other trials.

	Day 2 – Cycle		Day 4 - Run	
	<i>Pre-exercise</i>	<i>Post-exercise</i>	<i>Pre-exercise</i>	<i>Post-exercise</i>
<b>s-IgA Concentration (<math>\mu\text{g}\cdot\text{mL}^{-1}</math>)</b>				
LOW	162 $\pm$ 82	229 $\pm$ 147*	195 $\pm$ 87	229 $\pm$ 108*
HIGH	145 $\pm$ 78	243 $\pm$ 180*	134 $\pm$ 59	224 $\pm$ 136*
<b>Salivary Flow Rate (<math>\text{mL}\cdot\text{min}^{-1}</math>)</b>				
LOW	0.62 $\pm$ 0.35	0.50 $\pm$ 0.35 <sup>#</sup>	0.83 $\pm$ 0.49	0.64 $\pm$ 0.36 <sup>#</sup>
HIGH	0.74 $\pm$ 0.45	0.59 $\pm$ 0.55 <sup>#</sup>	0.80 $\pm$ 0.59	0.49 $\pm$ 0.27 <sup>#</sup>
<b>s-IgA Secretion Rate (<math>\mu\text{g}\cdot\text{min}^{-1}</math>)</b>				
LOW	98 $\pm$ 73	92 $\pm$ 49	165 $\pm$ 137 <sup>^</sup>	144 $\pm$ 116 <sup>^</sup>
HIGH	122 $\pm$ 136	126 $\pm$ 120	95 $\pm$ 59	101 $\pm$ 71

361 **Figure Caption List**

362

363 **Figure 1.** Overview of the randomized crossover design for elite triathletes undertaking two four-day  
364 experimental trials, inclusive of a 48 h breakdown of the carbohydrate periodization around key high  
365 intensity (HIT) and low intensity (LIT) sessions.

366

367 **Figure 2.** Concentrations of interleukin-6 (A and B) and hepcidin-25 (C and D) on days 2 and 4  
368 when adopting a sleep low (LOW) or sleep high approach (HIGH). \* Indicates a significant increase  
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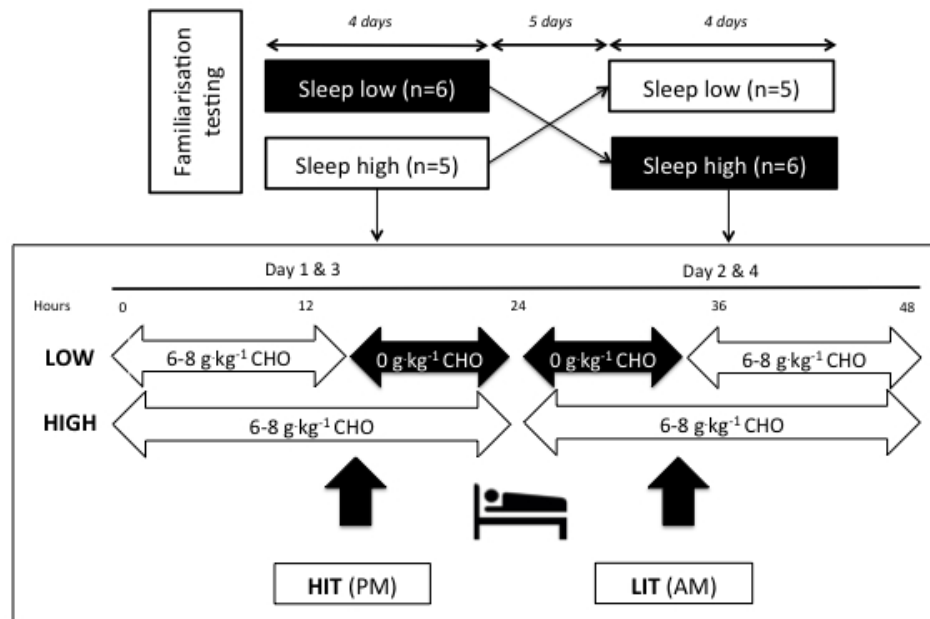


Figure 1. Overview of the randomized crossover design for elite triathletes undertaking two four-day experimental trials, inclusive of a 48 h breakdown of the carbohydrate periodization around key high intensity (HIT) and low intensity (LIT) sessions.

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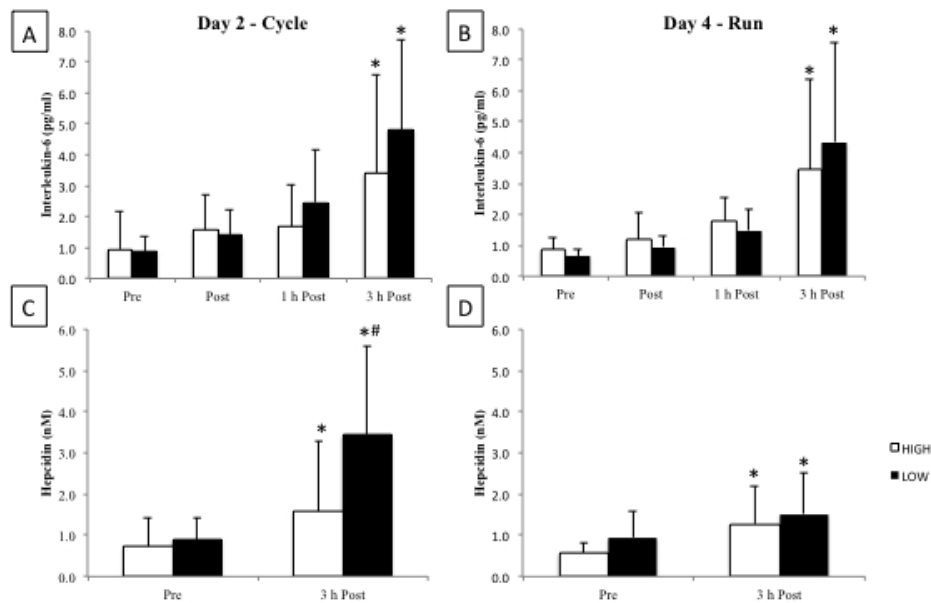


Figure 2. Concentrations of interleukin-6 (A and B) and hepcidin-25 (C and D) on days 2 and 4 when adopting a sleep low (LOW) or sleep high approach (HIGH). \* Indicates a significant increase from pre-exercise. # Indicates a significantly greater response compared both the HIGH condition and running trials. Data presented as Mean ( $\pm$ SD).

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